

## DATA EVALUATION RECORD

PP450 (FLUTRIAFOL TECHNICAL)

Study Type: OPPTS 870.3100 [§82-1a], Subchronic Oral Toxicity Study in Rats

Work Assignment No. 5-1-151 A; formerly 4-1-151 A (MRIDs 47090345 and 47090344)

Prepared for

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### Disclaimer

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OPPTS 870.3100/ DACO 4.3.1/ OECD 408

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Date: 8/21/09

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DATA EVALUATION RECORD
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**STUDY TYPE**: 90-Day Oral Toxicity [feeding - rats]; OPPTS 870.3100 [§82-1a] (rodent);  
OECD 408.

**PC CODE**: 128940**DP BARCODE**: 340368**TEST MATERIAL (PURITY)**: PP450 (93% a.i.)

**SYNONYMS**: Flutriafol technical;  $\alpha$ -(2-fluorophenyl)- $\alpha$ -(4-fluorophenyl)-1H-1,2,4-triazole-1  
ethanol

**CITATION**: Pigott, G.H. (1982) PP450 (Flutriafol technical): 90-Day feeding study in rats.  
Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park,  
Cheshire, UK. Laboratory Study No.: PR0432, Laboratory Report No.  
CTL/P/744, October 21, 1982. MRID 47090345. Unpublished.

Doe, J.E. (1982) PP450 (Flutriafol technical): 28-Day feeding study in rats.  
Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park,  
Cheshire, UK. Laboratory Study No.: PR0429, September 23, 1982. MRID  
47090344. Unpublished.

**SUBMITTER/SPONSOR**: Cheminova, Inc. (originally sponsored by Imperial Chemical  
Industries, Plc), 1600 Wilson Blvd, Suite 700, Arlington, VA

**EXECUTIVE SUMMARY**: In a subchronic oral toxicity study (MRID 47090344), PP450  
(93% a.i.; Batch No. P10) was administered to 20 Wistar rats/sex/dose in the diet at dose levels  
of 0, 20, 200, or 2000 ppm (calculated to be 0, 1.5, 14 and 158 mg/kg bw/day in males, and, 0,  
1.6, 22 and 145 mg/kg/day in females) for 90 days.

No treatment-related effects were noted on mortality, clinical signs of toxicity, ophthalmoscopic  
examinations, urinalysis, or gross pathology at any dose in either sex.

At 2000 ppm, body weight gains were decreased ( $p < 0.01$ ) throughout the study by 15-62% in  
both sexes. Food consumption was decreased ( $p < 0.05$ ) by 7-21% in the males (Weeks 1, 3, 5, 8,  
10, and 12) and 9-35% in the females (throughout the study). Total (Weeks 1-13) food  
consumption was decreased ( $p < 0.01$ ) by 7-19% in both sexes. At 200 ppm, sporadic decreases

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( $p < 0.05$ ) of 5-12% were noted in food consumption and overall food consumption was decreased by 6-7% in both sexes. At 20 ppm, sporadic decreases ( $p < 0.05$ ) in food consumption of 4-12% in was observed in both sexes.

Slight anemia was noted at 2000 ppm as indicated by decreases ( $p < 0.01$ ) in the following parameters: (i) hemoglobin ( $\downarrow$ 4-7%) at Weeks 4 and 13; (ii) hematocrit ( $\downarrow$ 5%) at Week 13; (iii) mean corpuscular volume ( $\downarrow$ 3%) at Week 13; (iv) mean corpuscular hemoglobin ( $\downarrow$ 3-4%) at Weeks 4 and 13 and (v) mean corpuscular hemoglobin concentration at Weeks 4 and 13 ( $\downarrow$ 1-3%). The kaolin-cephalin time was decreased ( $\downarrow$ 13%) at terminal sacrifice. APDM activity was increased ( $p < 0.05$ ) by 22-27% in both sexes, triglycerides were decreased ( $p < 0.01$ ), and cholesterol was increased ( $p < 0.01$ ) at Weeks 4 and 13 in both sexes.

The target organ was the liver. At 200 ppm, the absolute and adjusted liver weights were increased ( $p < 0.05$ ) in females by 5-8% at this dose. At 2000 ppm, increases ( $p < 0.01$ ) in absolute and adjusted for body weight liver weights were observed in both sexes. Increased incidence (# affected/40) of hepatocyte vacuolation (fatty change) was noted in 25 treated animals vs. 5 controls. Centrilobular hypertrophy (25 treated vs. 0 controls) with associated proliferation of smooth endoplasmic reticulum and elevated aminopyridine-N-demethylase (APDM) activity was also observed in both sexes at this dose. Smooth endoplasmic reticulum proliferation in the liver was increased ( $p < 0.01$ ) in the males.

**The LOAEL is 2000 ppm (158/145 mg/kg bw/day in males/females) based on decreased body weight gain; decreased food consumption and liver toxicity (increased absolute and adjusted liver weights, increased endoplasmic reticulum proliferation in the males, and increased APDM activity). The NOAEL is 200 ppm (14/22 mg/kg bw/day in males/females).**

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3100a; OECD 408) for a subchronic oral toxicity study in the rat.

**COMPLIANCE:** Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

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## I. MATERIALS AND METHODS

### A. MATERIALS

#### 1. Test material:

PP450

**Description:**

White powder

**Batch #:**

P10

**Purity:**

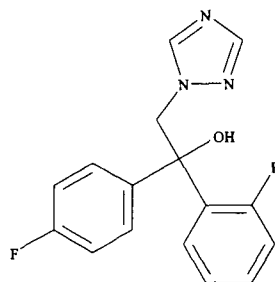
93% a.i.

**Stability:**

The test material was shown to be stable in the diet for up to 10 weeks at room temperature.

**CAS # of TGA:**

76674-21-0

**Structure:**

#### 2. Vehicle: Diet

#### 3. Test animals

**Species:**

Rat

**Strain:**

Wistar-derived

**Age/mean weight at study initiation:**

Approximately 4-5 weeks/128.5-133.7 g males and 114.7-118.4 g females

**Source:**

Animal Breeding Unit, Imperial Chemical Industries PLC (Alderley Park, Cheshire, UK)

**Housing:**

4/cage by sex in suspended stainless steel and wire mesh cages

**Diet:**Porton Combined Diet (Special Diet Services, Witham, Essex, UK), *ad libitum*; except during urine collection period**Water:**Filtered mains water, *ad libitum*; except during urine collection period**Environmental conditions****Temperature:** 17-32°C**Humidity:** 32-50%**Air changes:** At least 15/hr**Photoperiod:** 12 hrs dark/ 12 hrs light**Acclimation period:** 10 days

### B. STUDY DESIGN

#### 1. In-life dates: Start: January, 1982

End: April, 1982

#### 2. Animal assignment: The animals were randomly assigned, stratified by litter, to the test groups presented in Table 1.

TABLE 1. Study design <sup>a</sup>				
Test group	Dose (ppm)	Intake (mg/kg/day) <sup>b</sup>	# Males	# Females
Control	0	0	20	20
Low	20	1	20	20
Mid	200	10	20	20
High	2000	100	20	20

a Data were extracted from page 15 of MRID 40790345.

b Approximate value calculated by reviewers using the conversion factor of 1 ppm=0.05 mg/kg/day (obtained from Subdivision F guidelines)

3. **Dose-selection rationale:** The doses in the current study were based upon the results of a previously conducted 28-day feeding study (MRID 47090344) which is summarized in Appendix I to this DER.
4. **Treatment preparation, administration, and analysis:** Dietary formulations were prepared approximately monthly by adding the appropriate amount of PP450 (adjusted for purity) to 50 kg batches of ground (20 µm) diet. Homogeneity was evaluated at the low concentration (20 ppm) from the first batch of diet. Homogeneity was previously demonstrated at levels of 100 ppm and above in the 28-day study (MRID 47090344), and stability in the diet for up to 10 weeks at room temperature was also demonstrated in the 28-day study. Concentration analyses were performed on all dose levels from each batch prepared during the study.

## **Results**

**Homogeneity analysis (range as mean % of nominal):** 93.5-106%

**Stability analysis (range as % of initial value):**

Room temperature for up to 10 weeks: 95.5-98.0%

**Concentration analysis (range as mean % of nominal):**

Concentration (ppm)	Mean % of nominal
0	0
20	94.0-104.5
200	91.3-95.2
2000	94.8-105.3

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable.

**5. Statistics:** The following statistical procedures were used:

Parameter	Statistical Test
Body weight gain, weekly food consumption, total food consumption, food utilization, hematology, and liver aminopyrine-N-demethylase activity	Analysis of variance (ANOVA)
Organ weight	Analysis of variance (ANOVA) and analysis of covariance (ANCOVA) on final body weight
Clinical chemistry and urinalysis	Analysis of covariance (ANCOVA) on pre-experimental values (except for alkaline phosphatase activity)

If a significant difference was detected among the groups using the methods above, pairwise comparisons of treated groups with controls were conducted using a two-sided Student's t-test based on the error mean square in the analysis. Significance was denoted at  $p < 0.05$  or  $p < 0.01$ . Data should be tested to ensure that the assumptions of normal distribution and homogeneous variances are met prior to proceeding with parametric analyses. Otherwise, the reviewers consider the statistical methods to be appropriate.

**C. METHODS****1. Observations**

- a. **Cageside observations:** Animals were observed at least once daily for mortality and signs of toxicity.
- b. **Clinical examinations:** Detailed clinical observations were performed weekly throughout the study at the same time that body weights were recorded.
- c. **Neurological evaluations:** Neurological evaluations were not performed; however, acute (MRID 47090408) and subchronic (MRID 47090410) neurotoxicity studies were reviewed concurrently.
2. **Body weight:** All animals were weighed on Day 1 (prior to initiation of treatment), weekly during the study, and at termination.
3. **Food consumption and utilization:** Food consumption was recorded for each cage throughout the study, and mean food consumption (g/animal/day) was reported for each week. Total (Weeks 1-13) food consumption was also reported. Food utilization (g food/g bw) was calculated for Weeks 1-4, 5-8, 9-13, and overall (1-13).
4. **Ophthalmoscopic examination:** Ophthalmoscopic examinations were performed on all control and 2000 ppm animals during the week prior to scheduled sacrifice.
5. **Hematology and clinical chemistry:** Blood samples were collected from 10 rats/sex/dose via the tail vein (one day prior to initiation of dosing and at Week 4) or cardiac puncture (at termination) for hematological analyses. Additional blood samples were collected from 10 rats/sex/dose (not selected for hematology) via the tail vein at one day prior to initiation of

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dosing, at Week 4, and at termination for clinical chemistry analyses. The following CHECKED (X) parameters were examined:

**a. Hematology**

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*	X	Erythrocyte morphology
	(Activated partial thromboplastin time)		
X	(Kaolin-cephalin time)		
X	(Prothrombin time)		

\* Recommended for 90-day oral rodent studies based on Guideline 870.3100

**b. Clinical chemistry**

ELECTROLYTES		OTHER	
	Calcium	X	Albumin*
	Chloride		Creatinine*
	Magnesium	X	Urea nitrogen*
	Phosphorus	X	Total cholesterol*
	Potassium*		Globulins
	Sodium*	X	Glucose*
	<b>ENZYMES (more than 2 hepatic enzymes)</b>		Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total protein*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		Albumin/globulin
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

\* Recommended for 90-day oral rodent studies based on Guideline 870.3100

6. **Urinalysis:** Urine was collected from 10 fasted (food and water) rats/sex/dose (same animals used for clinical chemistry evaluations) one week prior to initiation of dosing, at Week 4, and one week prior to termination. The animals were individually placed in metabolism cages, and urine was collected over an 18 hour period. The following CHECKED (X) parameters were examined:

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	Appearance/color*	X	Glucose
X	Volume*	X	Ketones
X	Specific gravity/osmolality*		Bilirubin
X	pH*		Blood/blood cells*
	Sediment (microscopic)		Nitrate
X	Protein*	X	Urobilinogen

\* Optional for 90-day oral rodent studies

7. **Sacrifice and pathology:** At the end of the treatment period, all animals were weighed and sacrificed via exsanguination under halothane anesthesia. The following CHECKED (X) tissues from the main study animals were collected for histological examination. The (XX) organs, additionally, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	X	Thymus*+		<b>GLANDULAR</b>
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		<b>UROGENITAL</b>		Lacrimal gland
X	Colon*	XX	Kidneys*+		Parathyroid*
	Rectum*	X	Urinary bladder*	X	Thyroid*
XX	Liver*+	XX	Testes*+		<b>OTHER</b>
	Gall bladder (not rat)*	X	Epididymides*+	X	Bone (sternum and/or femur)
	Bile duct (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicles*	X	Skin*
	<b>RESPIRATORY</b>	XX	Ovaries*+	X	All gross lesions and masses*
	Trachea*	X	Uterus (with cervix)*+	X	Harderian gland
XX	Lung*	X	Mammary gland*		
	Nose*				
	Pharynx*				
X	Larynx*				

\* Recommended for 90-day oral rodent studies based on Guideline 870.3100

+ Organ weights required for rodent studies.

All tissues from the control and 2000 ppm animals, as well as the liver, kidneys, heart, lungs, ovaries, uterus, testes, epididymides, prostate, seminal vesicles, and gross lesions/masses from all groups, were routinely processed, embedded in paraffin, sectioned (5µm), stained with hematoxylin and eosin, and examined microscopically.

At termination, an 8 mm cube of liver tissue collected from the right median lobe of 6 rats/sex/dose. The sample was fixed in 3% (v/v) aqueous buffered glutaraldehyde, and 1 mm slices from the outside of the cube were post-fixed in 1% (w/v) buffered osmium tetroxide

and dehydrated in graded acetones prior to embedding in Araldite resin. The samples were sectioned (1  $\mu$ m), stained with toluidine blue, and examined with a light microscope in order to select centrilobular areas for electron microscopy. Ultra thin sections (70-90 nm) were cut, stained with uranyl acetate and lead citrate, and examined for evidence of proliferation of smooth endoplasmic reticulum (SER). Peroxisomes were also assessed in the females.

Unfixed liver samples were taken from 10 rats/sex/dose (including those selected for electron microscopy) for measurement of aminopyrine-N-demethylase (APDM) activity using the method of Mazel (1971).

## II. RESULTS

### A. OBSERVATIONS

1. **Mortality:** At 20 ppm, one male was found dead during Week 5 (no cause of death was established). Additionally, one male at this dose accidentally died during tail bleeding at Week 4. All other animals survived to scheduled sacrifice.
2. **Clinical signs:** It was stated that no treatment-related effects were observed on clinical signs during cage-side or detailed clinical observations. However, no summary or individual data were provided.

- B. **BODY WEIGHT AND WEIGHT GAIN:** At 2000 ppm, body weights were decreased throughout the study by 12-15% in the males and by 8-13% in the females (Table 2). The minor decrease in body weight ( $\downarrow$ 2%) noted in the 200 ppm males at Week 13 was biologically insignificant. At 2000 ppm, body weight gains were decreased ( $p \leq 0.01$ ) throughout the study by 15-62% in the males and by 18-46% in the females (Table 3). The minor decrease in body weight gain ( $\downarrow$ 8%,  $p < 0.01$ ) noted in the 200 ppm females at Week 4 was transient.

TABLE 2. Mean ( $\pm$ SD) body weight (g) in rats treated with PP450 in the diet for up to 13 weeks. <sup>a</sup>				
Interval (Weeks)	Dose (ppm)			
	0	20	200	2000
<b>Males</b>				
1	133.7 $\pm$ 16.7	130.0 $\pm$ 14.9	128.5 $\pm$ 12.8	131.7 $\pm$ 13.9
4	291.5 $\pm$ 21.2	292.2 $\pm$ 29.3	288.6 $\pm$ 13.3	248.0 $\pm$ 23.1 ( $\downarrow$ 15)
7	380.7 $\pm$ 27.9	380.7 $\pm$ 37.1	379.2 $\pm$ 28.7	335.1 $\pm$ 26.3 ( $\downarrow$ 12)
13	490.2 $\pm$ 41.3	489.5 $\pm$ 53.2	481.7 $\pm$ 35.8 ( $\downarrow$ 2)	431.8 $\pm$ 38.7 ( $\downarrow$ 12)
<b>Females</b>				
1	114.7 $\pm$ 12.4	116.8 $\pm$ 12.1	118.4 $\pm$ 13.8	115.2 $\pm$ 11.9
4	193.8 $\pm$ 15.3	196.3 $\pm$ 15.8	197.7 $\pm$ 14.9	178.6 $\pm$ 13.3 ( $\downarrow$ 8)
7	236.9 $\pm$ 20.1	238.4 $\pm$ 13.6	237.6 $\pm$ 15.6	214.7 $\pm$ 11.7 ( $\downarrow$ 9)
13	277.4 $\pm$ 24.0	278.2 $\pm$ 13.3	277.4 $\pm$ 20.0	241.8 $\pm$ 14.0 ( $\downarrow$ 13)

- a Data were extracted from Appendix A on pages 128-135 of MRID 47090345; n=18-20. Percent difference from control (calculated by reviewer) is presented parenthetically.

TABLE 3. Mean cumulative body weight gains (g) in rats treated with PP450 in the diet for up to 13 weeks. <sup>a</sup>				
Interval (Weeks)	Dose (ppm)			
	0	20	200	2000
<b>Males</b>				
1	54.4	54.7	54.9	20.6** (↓62)
4	194.2	196.0	196.7	151.4** (↓22)
7	271.2	274.7	275.7	226.9** (↓16)
13	360.8	367.2	360.9	308.2** (↓15)
<b>Females</b>				
1	34.1	33.4	32.6	18.5** (↓46)
4	101.8	98.8	93.9** (↓8)	81.9** (↓20)
7	132.5	134.1	129.6	107.0** (↓19)
13	156.8	161.6	157.9	127.8** (↓18)

a Data were extracted from Tables 4 and 5 on pages 32-33 of MRID 47090345; n=18-20. Percent difference from control (calculated by reviewers) is presented parenthetically. Standard deviations were not provided

\*\* Statistically significantly different from controls at  $p \leq 0.01$

**C. FOOD CONSUMPTION:** Food consumption was generally decreased in the treated groups compared to controls in both sexes throughout the study. At 2000 ppm, food consumption was decreased ( $p < 0.05$ ) by 7-21% in the males at Weeks 1, 3, 5, 8, 10, and 12 and by 9-35% in the females throughout the study (Table 4). At 200 ppm, food consumption was decreased ( $p < 0.05$ ) by 8-10% in the males at Weeks 3, 5, and 12 and by 5-12% in the females at Weeks 2-4, 6-7, and 9. At 20 ppm, food consumption was decreased ( $p < 0.05$ ) by 7-9% in the males at Weeks 3 and 5 and by 4-12% in the females at Weeks 2-4, 6, and 9. In all treatment groups, total (Weeks 1-13) food consumption was dose-dependently decreased ( $p \leq 0.05$ ) by 4-7% in the males and by 6-19% in the females. The statistically significant ( $p < 0.05$ ) differences in food utilization noted in the 2000 ppm males and the 20 ppm females were considered biologically insignificant.

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**TABLE 4. Mean food consumption (g/animal/day) and food utilization (g food/g bw) in rats treated with PP450 in the diet for up to 13 weeks. <sup>a</sup>**

Interval (Weeks)	Dose (ppm)			
	0	20	200	2000
<b>Males</b>				
1	22.3	21.9	20.3	17.6** (↓21)
3	26.7	24.3** (↓9)	24.1** (↓10)	22.3** (↓16)
5	27.3	25.3** (↓7)	24.7** (↓10)	25.4** (↓7)
12	25.2	26.0	23.2* (↓8)	23.4* (↓7)
13	22.3	22.2	22.5	23.5
<b>Total (Weeks 1-13)</b>	2327.3	2237.0* (↓4)	2182.2** (↓6)	2159.4** (↓7)
<b>Overall (Weeks 1-13) utilization</b>	6.46	6.11	6.07	7.02* (↑9)
<b>Females</b>				
1	17.2	16.6	15.8	13.3** (↓23)
2	18.3	16.4** (↓10)	16.4** (↓10)	15.9** (↓13)
3	18.8	16.6** (↓12)	17.5* (↓7)	16.2** (↓14)
4	19.8	17.9** (↓10)	17.4** (↓12)	16.6** (↓16)
5	18.3	18.5	17.9	16.6* (↓9)
8	20.1	18.3	18.6	13.1** (↓35)
9	18.2	17.4* (↓4)	16.7** (↓5)	14.2** (↓22)
13	15.6	15.1	15.1	13.2** (↓15)
<b>Total (Weeks 1-13)</b>	1692.8	1582.9** (↓6)	1566.5** (↓7)	1372.8** (↓19)
<b>Overall (Weeks 1-13) utilization</b>	10.66	9.82* (↓8)	9.98	10.81

a Data were extracted from Tables 6-9 on pages 34-37 of MRID 47090345; n=18-20. Percent difference from control (calculated by reviewers) is presented parenthetically. Standard deviations were not provided.

\* Statistically significantly different from controls at  $p \leq 0.05$

\*\* Statistically significantly different from controls at  $p \leq 0.01$

**D. OPHTHALMOSCOPIC EXAMINATION:** No treatment-related ocular lesions were observed at Week 13 in either sex.

**E. BLOOD ANALYSES**

1. **Hematology:** Slight anemia was noted at 2000 ppm as indicated by decreases ( $p \leq 0.01$ ) in the following parameters: (i) hemoglobin (↓4-7%) at Weeks 4 and 13; (ii) hematocrit (↓5%) at Week 13; (iii) mean corpuscular volume (↓3%) at Week 13; (iv) mean corpuscular hemoglobin (↓3-4%) at Weeks 4 and 13; (v) mean corpuscular hemoglobin concentration at Weeks 4 and 13 (↓1-3%), and (vi) kaolin-cephalin time (↓13%) at terminal sacrifice (Tables 5 a, b). Although these effects were considered to be related to treatment, they were minor and not considered to be adverse during the study period. All other statistically significant differences noted were considered incidental and unrelated to treatment because they were minor and/or were not dose-dependent.

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TABLE 5a. Hematological changes in male rats <sup>a</sup>				
Week	Dose (ppm)			
	0	20	200	2000
Hemoglobin (g/dL)				
0	12.82	13.18	13.18	12.85
4	15.79	16.21	15.80	15.16* (↓4)
13	15.51	15.57	15.43	14.65** (↓6)
RBC (x10 <sup>12</sup> /l)				
0	6.10	6.26	6.25	6.08
4	7.79	7.99	7.80	7.59
13	8.69	8.72	8.66	8.30* (↓4)
Hematocrit				
0	0.361	0.368	0.368	0.369
4	0.426	0.432	0.428	0.418
13	0.438	0.434	0.434	0.419* (↓4)
MCV (fl)				
0	59.9	59.4	59.5	59.8
4	54.4	54.1	54.8	55.1
13	50.9	50.3	50.7	50.9
MCH (pg)				
0	21.32	21.32	21.41	21.36
4	19.88	19.88	19.90	19.58
13	17.87	17.86	17.82	17.70
MCHC (g/dl)				
0	35.84	36.12	36.16	35.99
4	36.46	36.74	36.21	35.52** (↓3)
13	35.77	36.23** (↑1)	35.92	35.35** (↓1)

Data obtained from pages 939, 41, 43, 45, 47 and 49 of MRID 47090345.

<sup>a</sup> Standard d Standard deviations were not provided; values in parentheses are percent difference from control, calculated by the reviewer.

\*Significantly different (p≤0.05) from the control.

\*\*Significantly different (p≤0.01) from the control.

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OPPTS 870.3100/ DACO 4.3.1/ OECD 408

TABLE 5b. Hematological changes in female rats <sup>a</sup>				
Week	Dose (ppm)			
	0	20	200	2000
Hemoglobin (g/dL)				
0	12.60	12.97	12.31	12.46
4	15.87	16.41	16.33	15.25* (↓4)
13	15.02	14.97	14.79	13.89** (↓8)
RBC (x10 <sup>12</sup> /l)				
0	6.06	6.11	5.84	6.01
4	7.71	7.93	7.89	7.78
13	7.94	7.95	7.75	7.88
Hematocrit				
0	0.354	0.365	0.346	0.350
4	0.434	0.443	0.445	0.422
13	0.433	0.432	0.430	0.407** (↓6)
MCV (fl)				
0	58.8	60.0	59.6	58.3
4	56.3	55.8	56.4	54.2** (↓4)
13	54.8	54.7	55.7	51.7** (↓6)
MCH (pg)				
0	20.73	21.18	21.10	20.69
4	20.24	20.30	20.31	19.27** (↓5)
13	18.94	18.82	19.10	17.60** (↓7)
MCHC (g/dl)				
0	35.22	35.20	35.26	35.26
4	35.89	36.26	35.98	35.44* (↓1)
13	34.96	34.97	34.72	34.46** (↓1)

Data obtained from pages 939, 41, 43, 45, 47 and 49 of MRID 47090345.

<sup>a</sup> Standard deviations were not provided; values in parentheses are percent difference from control, calculated by the reviewer.

\*Significantly different (p≤0.05) from the control.

\*\*Significantly different (p≤0.01) from the control.

2. **Clinical chemistry:** At 2000 ppm, triglycerides were decreased (p<0.01) by 38-41% at Weeks 4 and 13, and cholesterol was increased (p<0.01) by 113% at Week 4 and 12% at Week 13 (Table 6). All other statistically significant differences noted were considered incidental and unrelated to treatment because they were minor, sporadic, transient, and/or were not dose-dependent.

TABLE 6. Selected mean clinical chemistry parameters in rats treated with PP450 in the diet for up to 13 weeks. <sup>a</sup>					
Parameter		Dose (ppm)			
		0	20	200	2000
Triglycerides (mg/100 mL)	Week -1	83	76	84	82
	Week 4	143	135	128	89** (↓38)
	Week 13	145	167	144	85** (↓41)
Cholesterol (mg/100 mL)	Week -1	49.6	47.2	49.5	50.6
	Week 4	42.2	44.3	44.5	51.8** (↑113)
	Week 13	42.5	43.8	47.1* (↑11)	47.7** (↑12)

<sup>a</sup> Data were extracted from Tables 38 & 40 on pages 66 & 68 of MRID 47090345; n=19-20. Percent difference from control (calculated by reviewers) is presented parenthetically. Standard deviations were not provided.

\* Significantly different from the controls at p&lt;0.05

\*\* Significantly different from the controls at p&lt;0.01

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In both sexes, aminopyridine-N-demethylase (APDM) activity was increased ( $p < 0.05$ ) by 135-185% at 2000 ppm and to a lesser degree ( $\uparrow 22$ -27%) at 200 ppm (Table 7).

TABLE 7. Mean aminopyridine-N-demethylase (APDM) activity in rats treated with PP450 in the diet for 13 weeks. <sup>a</sup>				
Dose (ppm)				
0	20	200	2000	
Males				
30.3	29.3	38.4* ( $\uparrow 27$ )	71.3** ( $\uparrow 135$ )	
Females				
16.3	16.3	19.9* ( $\uparrow 22$ )	46.5** ( $\uparrow 185$ )	

a Data were extracted from Table 72 on page 100 of MRID 47090345; n=9-10. Percent difference from control (calculated by reviewers) is presented parenthetically. Standard deviations were not provided.

\* Statistically significantly different from controls at  $p < 0.05$

\*\* Statistically significantly different from controls at  $p < 0.01$

**F. URINALYSIS:** At 2000 ppm, the following differences ( $p < 0.05$ , unless otherwise stated) from controls were noted, but were not considered to be biologically important: (i) pH was decreased (5.96 treated vs. 6.16 controls) during Week 4; (ii) urine volume was decreased by 15-16% (not statistically significant); (iii) specific gravity was increased; and (iv) protein was decreased by 57% in the males at Week 4.

## G. SACRIFICE AND PATHOLOGY

1. **Organ weight:** At 2000 ppm, increases ( $p < 0.01$ ) in absolute ( $\uparrow 20$ -36%) and adjusted for body weight ( $\uparrow 29$ -53%) liver weights were observed in both sexes (Table 7). Additionally in the 200 ppm females, absolute and adjusted liver weights were increased ( $p < 0.05$ ) by 5-8%.

Other differences ( $p < 0.05$ ) in organ weights were minor, not dose-related, and/or organ toxicity was not corroborated by other clinical or pathological data.

TABLE 7. Mean absolute (g) and adjusted for body weight liver weights in rats treated with PP450 in the diet for 13 weeks. <sup>a</sup>				
Observation	Dose (ppm)			
	0	20	200	2000
Males				
Absolute	17.3	18.0	17.7	23.6** ( $\uparrow 36$ )
Adjusted	16.6	17.2	17.2	25.4** ( $\uparrow 53$ )
Females				
Absolute	9.2	9.7	9.9* ( $\uparrow 8$ )	11.0** ( $\uparrow 20$ )
Adjusted	9.1	9.4	9.6* ( $\uparrow 5$ )	11.7** ( $\uparrow 29$ )

a Data were extracted from Table 67 on page 95 of MRID 47090345; n=17-20. Percent difference from control (calculated by reviewers) is presented parenthetically. Standard deviations were not provided.

\* Statistically significantly different from controls at  $p < 0.05$

\*\* Statistically significantly different from controls at  $p < 0.01$

2. **Gross pathology:** No treatment-related gross lesions were noted.

3. **Microscopic pathology:** In the liver, increased incidence (# affected/20) of the following was noted at 2000 ppm (Table 8): centrilobular hypertrophy in the males (17 treated vs. 0 controls) and females (8 treated vs. 0 controls) and hepatocyte vacuolation (fatty change) in the males (19 treated vs. 3 controls) and females (6 treated vs. 2 controls).

TABLE 8. Selected microscopic findings (# affected) in the livers of rats treated with PP450 in the diet for 13 weeks. <sup>a</sup>				
Observation	Dose (ppm)			
	0	20	200	2000
<b>Males</b>				
Centrilobular hypertrophy	0	0	0	17
Hepatocyte vacuolation (fatty change)	3	4	6	19
<b>Females</b>				
Centrilobular hypertrophy	0	0	0	8
Hepatocyte vacuolation (fatty change)	2	1	2	6

a Data were extracted from Table 75 on page 105 of MRID 47090345; n=18-20.

Electron microscopic evaluation of the liver revealed an increase ( $p < 0.01$ ) in smooth endoplasmic reticulum proliferation as a percent of cytoplasm observed in the 200 and 2000 ppm males (41.8-50.6% treated vs. 28.9% controls) and in the 2000 ppm females (51.4% treated vs. 24.4% controls, Table 9).

TABLE 9. Mean ( $\pm$ SD) smooth endoplasmic reticulum (% cytoplasm) in hepatocytes of rats treated with PP450 in the diet for 13 weeks. <sup>a</sup>				
	Dose (ppm)			
	0	20	200	2000
<b>Males</b>				
	28.9 $\pm$ 5.0	27.8 $\pm$ 5.7	41.8 $\pm$ 5.0**	50.6 $\pm$ 9.2**
<b>Females</b>				
	24.4 $\pm$ 2.9	26.4 $\pm$ 4.1	27.6 $\pm$ 3.5	51.4 $\pm$ 6.4**

a Data were extracted from Table 73 on page 101 of MRID 47090345; n=6.

\*\* Statistically significantly different from controls at  $p < 0.01$

### III. DISCUSSION AND CONCLUSIONS

- A. **INVESTIGATORS CONCLUSIONS:** The investigator concluded that rats fed PP450 at 2000 ppm displayed reduced body weight gain and food consumption together with fatty change and hypertrophy of the liver which is regarded as typical of a response to a mild toxicant coupled with an adaptive response. The rats fed 200 ppm showed a reduced adaptive response and only the males displayed a minimal toxic response. The NOAEL was 20 ppm.
- B. **REVIEWER'S COMMENTS:** No treatment-related effects were noted on mortality, clinical signs of toxicity, ophthalmoscopic examinations, urinalysis, or gross pathology at any dose in either sex.

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The target organ was the liver. At 2000 ppm, increases ( $p < 0.01$ ) in absolute and adjusted for body weight liver weights were observed in both sexes. Increased incidence (# affected/40) of hepatocyte vacuolation (fatty change) was noted in 25 treated animals vs. 5 controls. Centrilobular hypertrophy (25 treated vs. 0 controls) with associated proliferation of smooth endoplasmic reticulum and elevated aminopyridine-N-demethylase (APDM) activity was also observed in both sexes at this dose. Additionally, triglycerides were decreased ( $p < 0.01$ ) and cholesterol was increased ( $p < 0.01$ ) at Weeks 4 and 13 in both sexes.

At 2000 ppm, body weights were decreased throughout the study by 12-15% in the males and 8-13% in the females. Body weight gains were decreased ( $p < 0.01$ ) throughout the study by 15-62% in both sexes. Food consumption was decreased ( $p < 0.05$ ) by 7-21% in the males (Weeks 1, 3, 5, 8, 10, and 12) and 9-35% in the females (throughout the study). Total (Weeks 1-13) food consumption was decreased ( $p < 0.01$ ) by 7-19% in both sexes. Slight anemia was noted at this dose as indicated by decreases ( $p < 0.01$ ) in the following parameters: (i) hemoglobin ( $\downarrow 4-7\%$ ) at Weeks 4 and 13; (ii) hematocrit ( $\downarrow 5\%$ ) at Week 13; (iii) mean corpuscular volume ( $\downarrow 3\%$ ) at Week 13; (iv) mean corpuscular hemoglobin ( $\downarrow 3-4\%$ ) at Weeks 4 and 13; (v) mean corpuscular hemoglobin concentration at Weeks 4 and 13 ( $\downarrow 1-3\%$ ), and (vi) kaolin-cephalin time ( $\downarrow 13\%$ ) at terminal sacrifice. Although these effects were considered to be related to treatment, they were minor and not considered to be adverse within the timeframe of the study.

At 200 ppm, sporadic decreases ( $p < 0.05$ ) of 5-12% were noted in food consumption and overall food consumption was decreased by 6-7% in both sexes. Additionally in the females, absolute and adjusted liver weights were increased ( $p < 0.05$ ) by 5-8% at this dose. APDM activity was increased ( $p < 0.05$ ) by 22-27% in both sexes, and smooth endoplasmic reticulum proliferation in the liver was increased ( $p < 0.01$ ) in the males.

At 20 ppm, findings were limited to sporadic decreases ( $p < 0.05$ ) in food consumption of 4-12% in both sexes.

**The LOAEL is 2000 ppm (158/145 mg/kg bw/day in males/females) based on decreased body weight gain; decreased food consumption and liver toxicity (increased absolute and adjusted liver weights, increased endoplasmic reticulum proliferation in the males, and increased APDM activity). The NOAEL is 200 ppm (14/22 mg/kg bw/day in males/females).**

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3100a; OECD 408) for a subchronic oral toxicity study in the rat.

**C. STUDY DEFICIENCIES:** The following deficiencies were noted, but do not change the conclusions of this DER:

- The thymus, epididymides, and uterus were not weighed.
- The rectum, trachea, nose, pharynx, and parathyroids were not collected for histology.
- No clinical signs or gross pathology data were presented.

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**APPENDIX I****28-Day Dietary Dose Range Finding Study in Rats**

Since this is a range-finding study, only a summary is provided to confirm the adequacy of the dose selection rationale used in the definitive subchronic oral (dietary) toxicity study in rats (MRID 47090345).

In a 28-day oral toxicity study (MRID 47090344), PP450 (97% a.i., Batch # P7) was administered in the diet to 8 Wistar rats/sex/group at dose levels of 0, 100, 300, 800, 2000, or 5000 ppm (equivalent to approximately 10, 30, 80, 200, and 500 mg/kg bw/day) for 28 days.

No treatment-related effects were observed on mortality or gross pathology.

Slight anemia was noted at in males at 2000 ppm and 5000 ppm and in females at 5000 ppm. Although these effects were considered to be related to treatment, they were largely minor and not considered to be adverse within the timeframe of the study. The MCHC was decreased in females at 300 ppm and above. White blood cell counts and lymphocyte counts were increased, and the kaolin-cephalin time decreased in females in the 2000 ppm and 5000 ppm groups. Females in the 5000 ppm group also had decreases in the hematocrit and hemoglobin levels. The erythrocyte count, hemoglobin levels and MCHC were significantly decreased, and the white blood cell count was significantly increased in males at 2000 ppm and 5000 ppm. The kaolin-cephalin time was significantly increased in male at 5000 ppm.

The target organ was the liver. At 100 ppm and above, liver weights were significantly increased in males and in females at 300 ppm and above. Elevated aminopyridine-N-demethylase (APDM) activity was observed in both sexes at 300 ppm and above. At 800 ppm and above, increases ( $p < 0.05$ ) in absolute and adjusted for body weight liver weights were observed in both sexes. Increased incidences (# affected/8 vs. 0 controls) of the following histopathological findings were observed: (i) fatty change at 2000 ppm and above (7-8/sex); (ii) centrilobular hypertrophy at 800 ppm and above in males (6-8) and at 2000 ppm and above in females (5-7); and (iii) hydropic degeneration at 2000 ppm and above in 2-3 males and at 5000 ppm in 5 females. Additionally in the 2000 ppm males, proliferation of smooth endoplasmic reticulum was observed. Additionally, triglycerides were decreased ( $p < 0.01$ ) in both sexes at 5000 ppm.

Additionally at 5000 ppm, the following clinical signs of toxicity were observed: thinness, hunched posture, piloerection, and hair loss (females). Body weight losses or reduced body weight gain were observed in both sexes throughout the study. Food consumption was decreased in both sexes.

At 2000 ppm, body weight gain was decreased predominantly in the males.

**The LOAEL is 2000 ppm (equivalent to approximately 200 mg/kg bw/day), based upon liver toxicity (increased weight, centrilobular hepatocellular hypertrophy, fatty change, hydropic degeneration, smooth endoplasmic reticulum proliferation, and increased aminopyrine-N-demethylase activity) in both sexes and decreased body weight gain and**

**food consumption in males. The NOAEL is 800 ppm (equivalent to approximately 80 mg/kg bw/day).**

This study is classified as **acceptable/non-guideline**.

**COMPLIANCE**: Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

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